5 LINKAGE ANALYSIS

The Delta linkage analysis focuses on the comparison of methylmercury concentrations in water and biota. The relationship has not previously been evaluated in the Delta, but statistically significant, positive correlations have been reported between aqueous methylmercury and aquatic biota elsewhere (Brumbaugh *et al.*, 2001; Foe *et al.*, 2002; Slotton *et al.*, 2003; Tetra Tech, 2005; Sveinsdottir and Mason, 2005), suggesting that methylmercury levels in water may be one of the primary factors determining methylmercury concentrations in fish. This linkage analysis develops a Delta-specific mathematical relationship between aqueous and biotic methylmercury concentrations. The relationship is used to determine an aqueous methylmercury goal that, if met, is predicted to produce safe fish tissue levels for both human and wildlife consumption (Chapter 4). The aqueous methylmercury goal is then used to allocate methylmercury reductions for within-Delta and tributary sources (Chapter 8).

The linkage analysis has three sections. The first section describes the available fish and aqueous methylmercury data. The second section illustrates the mathematical relationship between unfiltered water and largemouth bass methylmercury levels. The mathematical relationship is used to develop an unfiltered aqueous methylmercury goal of 0.06 ng/l that is protective of all humans and wildlife that consume Delta fish. The final section provides an alternate linkage using 0.45 µ filtered methylmercury water data. Results of these correlation-based linkages are comparable to results of more empirical linkage methods, such as the evaluation of Delta areas that currently achieve the implementation goal for largemouth bass, and the use of bioaccumulation factors to calculate an aqueous methylmercury goal.

5.1 Data Used in Linkage Analysis

Fish. Water and fish have not been sampled in the Delta for the specific purpose of developing a linkage analysis. As a result, there is an acceptable overlap for only a portion of the available fish and water data. This linkage analysis focuses on recently collected largemouth bass data for several reasons. First, largemouth bass was the only species systematically collected near many of the aqueous methylmercury sampling locations used to develop the methylmercury mass balance for the Delta (next section). Second, largemouth bass are piscivorous and have some of the highest mercury levels of any fish species evaluated in the Delta. Third, bass are abundant and widely distributed throughout the Delta. Fourth, bass have high site fidelity (Davis and Greenfield, 2002), making them useful bioindicators of spatial variation in mercury accumulation in the aquatic food chain. Finally, spatial trends across the Delta in standard 350-mm largemouth bass mercury levels are representative of spatial trends in the trophic level food group mercury levels (Section 4.7). Largemouth bass were collected from 19 locations in the Delta in August/September 1998, 26 locations in September/October 1999, and 22 locations in September/October 2000 (Davis *et al.*, 2000; Davis *et al.*, 2003; LWA, 2003). The year 2000 largemouth bass data were used in the linkage analysis because the exposure period of these fish had the greatest overlap with the available water data. Monthly water data were collected during the last eight months of the life of the fish. Figure 5.1 shows the aqueous and largemouth bass methylmercury sampling locations used in the linkage analysis. The mercury concentrations in standard 350-mm largemouth bass and the corresponding water data for each sampling location are presented in Table 5.1. Section 4.8 in Chapter 4 describes the method used to calculate standard 350-mm largemouth bass mercury concentrations.

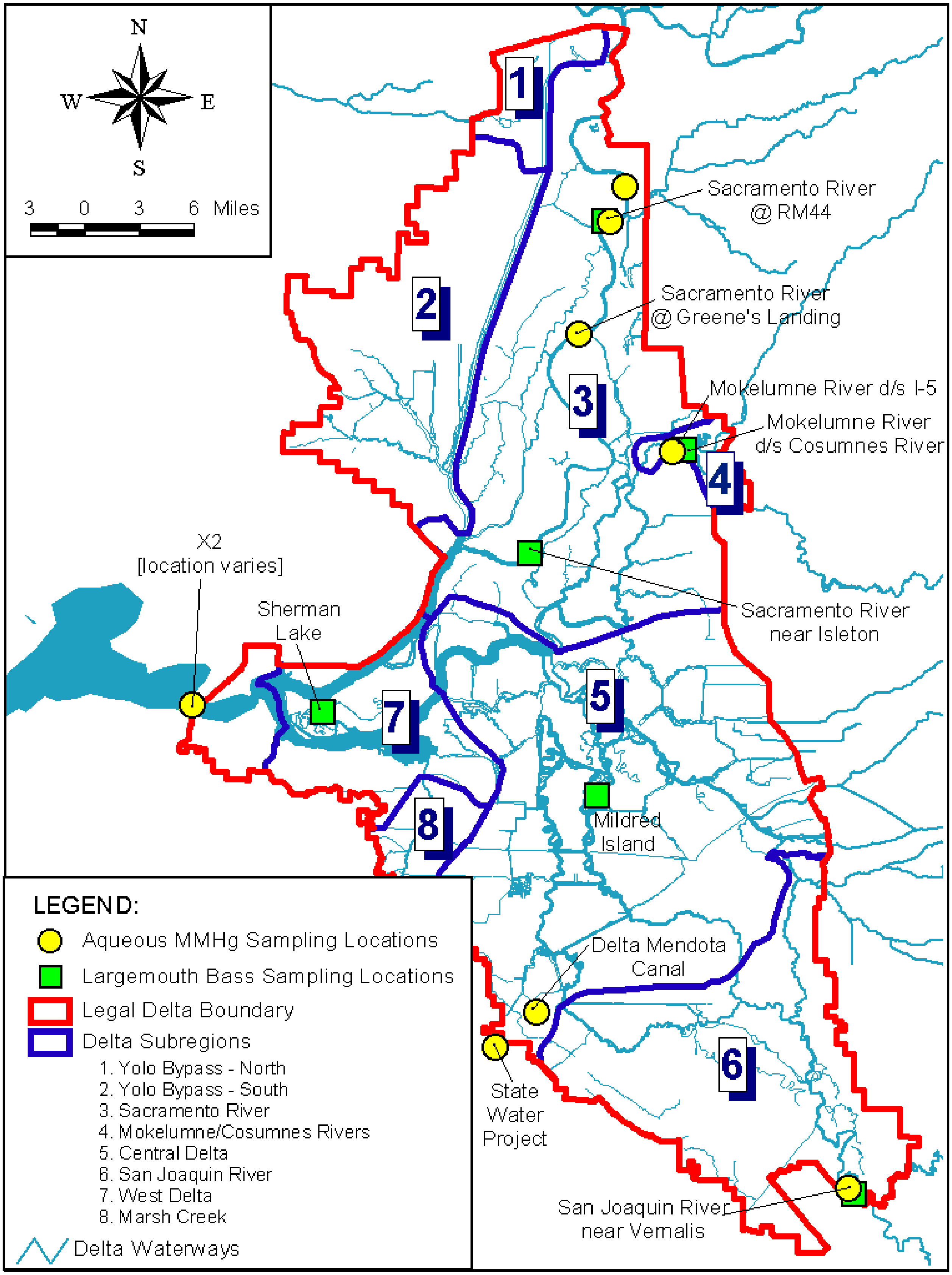

Figure 5.1: Aqueous and Largemouth Bass Methylmercury Sampling Locations Used in the Linkage Analysis.

Table 5.1: Fish and Water Methylmercury Values by Delta Subarea

	Delta Subarea (a)				
	Sacramento River	Mokelumne River	Central Delta	San Joaquin River	West Delta
FISH [Sampled in September/October 2000] (mg/kg)					
Standardized 350-mm Largemouth Bass	0.72	1.04	0.19	0.68	0.31
WATER [Sampled between March and October 2000] (ng/l)					
Average	0.120	0.140	0.055	0.147	0.087
Median	0.086	0.142	0.032	0.144	0.053
WATER [Sampled between March 2000 and April 2004] (ng/l)					
Annual Average	0.108	0.166	0.060	0.160	0.083
Annual Median	0.101	0.161	0.051	0.165	0.061
Cool Season Average (b)	0.137	0.221	0.087	0.172	0.106
Cool Season Median	0.138	0.246	0.077	0.175	0.095
Warm Season Average	0.094	0.146	0.050	0.156	0.075
Warm Season Median	0.089	0.146	0.040	0.162	0.055

(a) See Figure 5.1 for the location of each water and fish collection site.
(b) For this analysis, "cool season" is defined as November through February and "warm season" is defined as March through October.

Water. Unfiltered methylmercury water samples were collected periodically between March 2000 and April 2004 at multiple Delta locations (Figure 5.1, Appendix D). The monthly March-October 2000[25] subset of this data has the greatest overlap with the lifespan of the largemouth bass sampled in September/October 2000. The March-October 2000 and March 2000 to April 2004 data were pooled by Delta subarea to calculate monthly averages (Tables D.1 and D.2).[26] These values were used to estimate average and median methylmercury concentrations for the March-October 2000 period and annual and seasonal average and median concentrations for the March 2000 to April 2004 period (Table 5.1).[27]

[25] Coincidentally, March through October defines the season with warmer water temperatures. Aquatic biota may be more metabolically active and have a higher methylmercury bioaccumulation rate in summer. In addition, sulfate-reducing bacteria may have higher methylmercury production rates making this a critical bioaccumulation time period.

[26] The methylmercury concentrations for two periods – (a) March-October 2000 and (b) September 2000 to April 2004 – were compared at each sampling location in Figure 5.1 with a paired t-test to determine whether the mean concentrations for the two time periods were different. The tests indicated no significant difference ($P \leq 0.05$) for any location. Therefore, the data for March 2000 to April 2004 (a substantially larger database than that for March-October 2000) were also evaluated in the linkage analysis.

[27] Monthly averages were used to ensure that the seasonal and annual values were not biased by months with different sample sizes.

5.2 Bass/Water Methylmercury Regressions & Calculation of Aqueous Methylmercury Goal

The mercury concentrations in standard 350-mm largemouth bass for each Delta subarea were regressed against the average and median unfiltered aqueous methylmercury levels for the March to October 2000 and March 2000 to April 2004 periods to determine whether relationships might exist (Figure 5.2, Table 5.2, & Figure D.1 in Appendix D). The regressions were evaluated using linear, exponential, logarithmic, and power curves. Power curves provided the best fit, although all the regression types demonstrated a positive relationship between aqueous and biotic methylmercury concentrations. In each scenario described by Table 5.2, increasing the aqueous methylmercury concentration results in increasing fish tissue levels. All the scenarios were statistically significant ($P < 0.05$).

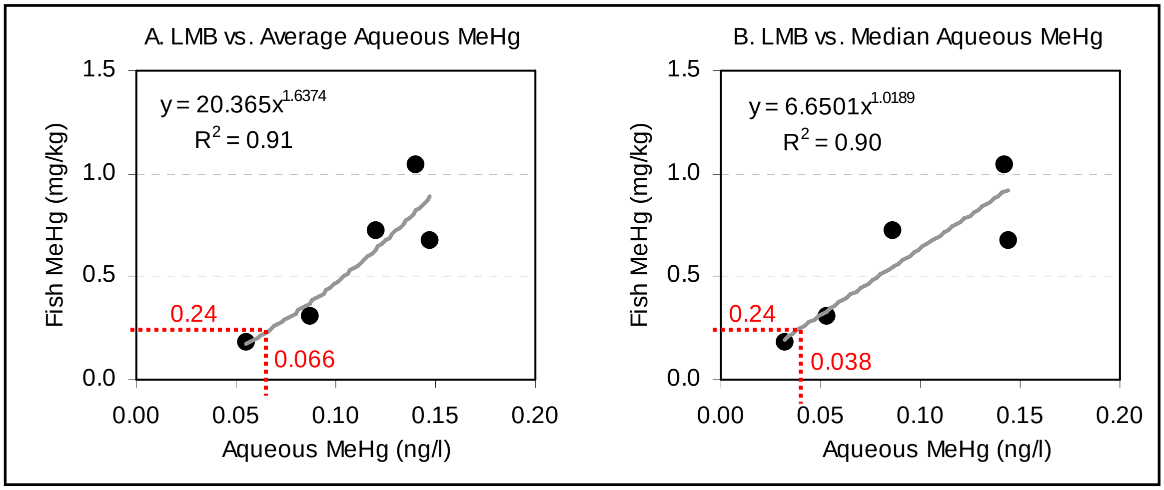

Figure 5.2: Relationships between Standard 350-mm Largemouth Bass Methylmercury & March to October 2000 Unfiltered Aqueous Methylmercury.
The proposed implementation goal for standard 350-mm largemouth bass is 0.24 mg/kg.

Table 5.2: Relationships between Methylmercury Concentrations in Water and Standard 350-mm Largemouth Bass

Aqueous MeHg Data Period	Scenario	Regression Equation	R^2 (a)	Aqueous MeHg Conc. (ng/l) Corresponding to LMB value of 0.24 mg/kg
1. March to October 2000	A. Average Aqueous MeHg	$y = 20.365x^{1.6374}$	0.91	0.066
	B. Median Aqueous MeHg	$y = 6.6501x^{1.0189}$	0.90	0.038
2. March 2000 to April 2004 - Annual -	A. Average Aqueous MeHg	$y = 14.381x^{1.51}$	0.88	0.066
	B. Median Aqueous MeHg	$y = 8.0903x^{1.1926}$	0.86	0.052
3. March 2000 to April 2004 - Cool Season -	A. Average Aqueous MeHg	$y = 17.795x^{1.8007}$	0.90	0.092
	B. Median Aqueous MeHg	$y = 8.8725x^{1.4347}$	0.92	0.081
4. March 2000 to April 2004 - Warm Season -	A. Average Aqueous MeHg	$y = 11.528x^{1.339}$	0.83	0.055
	B. Median Aqueous MeHg	$y = 6.8941x^{1.0723}$	0.85	0.044

(a) All R^2 values are statistically significant at $P<0.05$. Regression graphs are provided in Figure 5.2 and Appendix D.

The recommended implementation goal for methylmercury in the Delta is 0.24 mg/kg (wet weight) in a standard 350-mm largemouth bass (Chapter 4). Substitution of 0.24 mg/kg into the equations in Table 5.2

results in predicted average and median safe water methylmercury values that range from 0.04 to 0.09 ng/l. The lowest concentration is predicted by the regression based on median March to October 2000 water values (Scenario 1B) while the highest concentration is predicted by the regression based on average cool season water concentrations (Scenario 3A).

Staff recommends that **0.06 ng/l methylmercury in unfiltered water** be used as an **implementation goal** for the determination of load allocations (Chapter 8). This recommendation is based on Scenario 1A in Table 5.2 and incorporates an explicit margin of safety of about 10%. The goal could be applied as an annual average methylmercury concentration. Staff recommends this value because only the March to October 2000 period overlapped the lifespan of the largemouth bass analyzed for mercury body burden. Also, little is known about the seasonal exposure regime controlling methylmercury concentrations in aquatic biota. Therefore, an annual average was selected as it weights all seasons equally.

The linkage analysis for the Delta relies upon sequential correlations to determine the numerical aqueous methylmercury goal. A potential problem with the analysis is that each correlation has an associated error term. No attempt has been made to estimate these errors and propagate them from one correlation to the next when calculating the recommended aqueous methylmercury goal. There are two alternate, more empirical, approaches. The first approach is to compare existing largemouth bass and aqueous methylmercury levels to the proposed implementation goals. The average March-October 2000 methylmercury concentration in the Central Delta (0.055 ng/l, Table 5.1) is less than the proposed aqueous goal of 0.06 ng/l while concentrations in the West Delta (0.087 ng/l) are higher. Similarly, the methylmercury concentration in standard 350-mm bass in the Central Delta is 0.19 mg/kg while the concentration in the West Delta is 0.31 mg/kg (Table 4.10). The recommended implementation goal is 0.24 mg/kg in standard 350-mm largemouth bass. Therefore, empirical observations suggest that the "correct" aqueous methylmercury goal to achieve safe mercury levels in the various trophic level food groups must lie between 0.055 and 0.087 ng/l. If the aqueous methylmercury goal of 0.06 ng/l is attained in the Delta, then methylmercury concentrations in all trophic level food groups are predicted to fall within the safe tissue concentration range.

A second linkage approach that does not rely on the correlation between largemouth bass and water methylmercury concentrations to derive an implementation goal for water makes use of bioaccumulation factors (BAFs), an approach used in numerous USEPA-approved TMDLs across the country.[28] A BAF is the ratio of the concentration of a chemical in fish tissue to the concentration of the chemical in the water column. By definition, BAFs imply a linear relationship between methylmercury in the water column and in fish. Section D.2 in Appendix D describes the method used to develop BAF-based implementation goals for the Delta and its subregions using standard 350-mm largemouth bass and average aqueous methylmercury concentrations. The resulting safe aqueous methylmercury levels ranged from 0.029 to 0.069 ng/l, slightly less than but comparable to the safe levels produced using the regression-based approach. The similarity most likely occurs because both methods used the same fish and water data, and because the regression described in Figure 5.2(A) is nearly linear at low fish and water methylmercury levels. However, the regression-based method is preferred because it does not inherently assume a linear relationship between fish and water methylmercury levels.

The safe aqueous methylmercury concentrations predicted for the Delta are comparable to analysis results for Cache Creek and nationwide studies. Brumbaugh and others (2001) found in a national survey of

[28] Refer to: http://www.epa.gov/OWOW/tmdl/index.html.

106 stations from 21 basins that one-time unfiltered methylmercury water samples collected during the fall season were also positively correlated with largemouth bass tissue levels. An aqueous methylmercury concentration of 0.058 ng/l was predicted to produce three-year old largemouth bass[29] with 0.3 mg/kg mercury tissue concentration. In the Cache Creek watershed, an unfiltered methylmercury concentration of 0.14 ng/l corresponded with the production of 0.23 mg/kg mercury in large fish (CVRWQB, 2004). Predicted safe methylmercury water values for the Delta are bracketed by safe water concentrations determined by the national and Cache Creek studies.

Additional fish and methylmercury water studies that address uncertainties in the linkage analysis are planned. These include additional evaluations of standard 350-mm largemouth bass tissue concentrations at more locations in the Delta after multiple years of aqueous methylmercury data have been obtained. Studies also are planned to better determine the seasonal exposure regime when most of the methylmercury is sequestered in the aquatic food chain. The results of these studies may lead to future revisions in the proposed aqueous methylmercury goal.

5.3 Evaluation of a Filtered Aqueous Methylmercury Linkage Analysis

This section presents an alternate linkage analysis based on filter-passing[30] aqueous methylmercury data. Methylmercury concentrations in standard 350-mm largemouth bass for each Delta subarea (Table 5.1) were regressed against the average and median filtered aqueous methylmercury levels for March-October 2000 (Table 5.3 and Table D.3 in Appendix D). Figure 5.3 demonstrates that there is a statistically significant positive correlation between filter-passing aqueous and largemouth bass tissue methylmercury levels. However, average and median filter-passing methylmercury water values for the Central Delta and Western Delta, regions that define the lower end of the regression, are determined mainly by values lower than the method detection limit (0.022 ng/l). Furthermore, substitution of the recommended implementation goal of 0.24 mg/kg mercury for 350 mm largemouth bass in the equations in Figure 5.3 results in predicted average and median safe water values (0.016 ng/l and 0.010 ng/l, respectively) below the method detection limit. Similarly low levels resulted when the BAF-based linkage method was used (see Section D.2 in Appendix D). Staff does not recommend adoption of a methylmercury goal that is unquantifiable with present analytical methods.

Key points to consider for the linkage analysis are listed after Table 5.3 and Figure 5.3.

[29] 262-mm average length fish.

[30] Water samples were filtered using 0.45-micrometer capsule filters. Much of the methylmercury measured in filtered samples is colloidal (Choe, 2002). Hence the results are called “filter-passing” rather than “dissolved”.

Table 5.3: Average and Median Filtered Methylmercury Concentrations (ng/l) for March 2000 to October 2000 for Each Delta Subarea.

	Delta Subarea (a)				
	Sacramento River	Mokelumne River	Central Delta	San Joaquin River	West Delta
Average	0.120	0.140	0.055	0.147	0.087
Median	0.086	0.142	0.032	0.144	0.053

(a) See Figure 5.1 for the location of each water and fish collection site. See Tables D.4 and D.5 in Appendix D for raw data and monthly averages, upon which these average and median values are based.

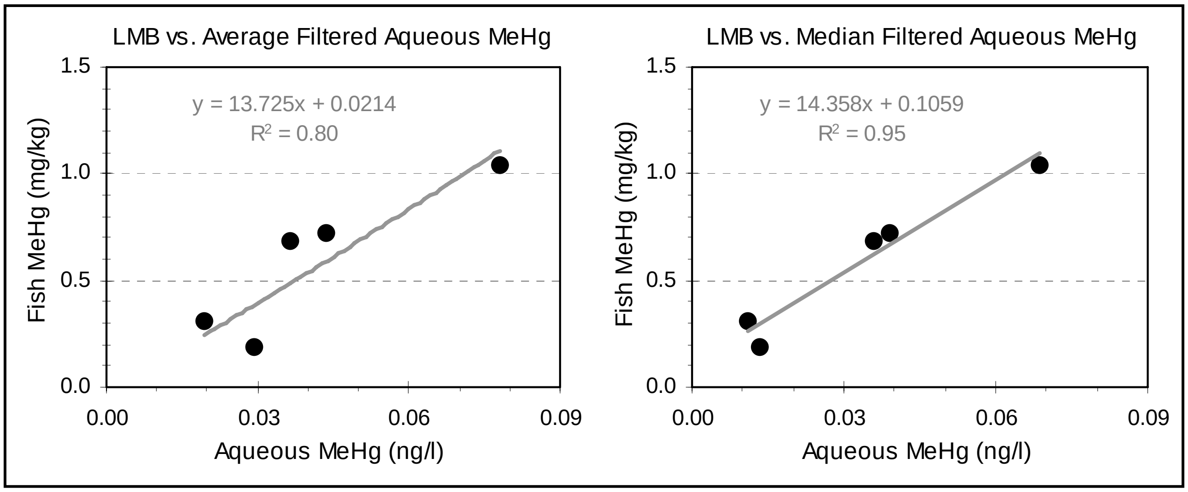

Figure 5.3: Relationships between Standard 350-mm Largemouth Bass Mercury Levels & March to October 2000 Filtered Aqueous Methylmercury.
The proposed implementation goal for standard 350-mm largemouth bass is 0.24 mg/kg.

Key Points

- Statistically significant mathematical relationships exist between unfiltered and filter-passing methylmercury concentrations in water and fish tissue.
- Based on the relationship between average March to October 2000 unfiltered methylmercury concentrations in water and methylmercury in standard 350-mm largemouth bass tissue, staff recommends an implementation goal for ambient Delta waters of 0.06 ng/l unfiltered methylmercury. The proposed goal incorporates an explicit margin of safety of about 10%. Staff recommends that the goal be applied as an annual average methylmercury concentration.
- More empirical linkage methods, such as the evaluation of Delta areas that currently achieve the implementation goal for largemouth bass and the use of bioaccumulation factors to calculate an aqueous methylmercury goal, predict safe aqueous methylmercury levels comparable to the correlation-based linkage method.